PCT/US97/23719

REC'D 12 FEB 1998
WIPO PCT

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

January 29, 1998

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *08/775,595*
FILING DATE: *December 31, 1996*

PRIORITY DOCUMENT

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

L. BLAND
Certifying Officer

08/774595

MAIL ROOM DEC 31 1996 PAT. & TRADEMARK OFF.

1053.00017

METHOD AND PHARMACEUTICAL COMPOSITION FOR REPLACING IRON LOSSES IN HEMODIALYSIS PATIENTS

TECHNICAL FIELD

The present invention relates to hemodialysis and more particularly to methods of supplementing dialysate solutions for the treatment of iron deficiency in hemodialysis patients.

BACKGROUND OF THE INVENTION

Patients with chronic renal failure suffer from anemia due to impaired production of erythropoietin (1). Clinical manifestations of chronic renal failure improve as uremia and volume overload were corrected by dialysis. However, anemia due to lack of erythropoietin becomes a major limiting factor in the functional well being of end stage renal disease patients.

Molecular cloning of the erythropoietin gene in 1985 (9) led to commercial production of recombinant erythropoietin, which was a major advance in the treatment of renal anemia (1,10). Erythropoietin therapy functions by stimulating red cell production and thereby iron utilization. With the use of erythropoietin therapy, transfusions are avoided in most chronic dialysis patients. However, accelerated iron utilization coupled with small but unavoidable loss of extra corporeal blood with hemodialysis lead and

increased gastrointestinal losses of iron to iron deficiency in almost all patients on long term maintenance dialysis.

Other factors that may contribute to an iron deficient state are restricted renal diet which may be deficient in iron, and iron absorption may be impaired by uremia per se. Administration of additional medication such as phosphate binders with food may also impair iron absorption. Therefore, iron deficiency has become a major problem in the maintenance of hemodialysis patients treated with erythropoietin.

Van Wyck et al. (14) have suggested that all renal patients with low to normal iron stores should prophylactically receive iron. Iron supplementation is accomplished most conveniently by the oral administration of iron one to three times a day.

A problem exists because oral iron is often not tolerated due to gastrointestinal side effects. Practical problems such as noncompliance, impaired absorption when taken with meals, and other factors are further combined with the problem of tolerating oral iron. . It is also ineffective due to impaired iron absorption. Macdougall et al. (16) also found a retarded response to recombinant human erythropoietin in hemodialysis patients on oral iron, which was corrected once iron was given intravenously. Schaefer and Schaefer (17) have recently demonstrated that only intravenous but not oral iron guarantees adequate marrow iron supply during the correction phase of recombinant erythropoietin therapy.

In Europe, iron is available for intravenous administration as iron dextran, iron saccharate and iron gluconate. In the United States, only iron dextran is approved for intravenous use and is widely used for this purpose in dialysis patients. However, there are controversies with regard to the dosage and frequency of injection.

On the one hand, intravenous iron therapy has several advantages over oral administration. Intravenous therapy overcomes both compliance problems and the low gastrointestinal tolerance often observed in patients on oral therapy. Schaefer and Schaefer (24) reported a 47% reduction in erythropoietin dose, when intravenous iron was given to iron deficient hemodialysis patients previously treated with oral iron. On the other hand, intravenous iron therapy does have risks and disadvantages. Anaphylactoid reactions have been reported in patients (26,27). Therefore, a test dose must be administered when parenteral iron therapy is first prescribed. Intravenous iron therapy can also cause hypotension, loin and epigastric pain during dialysis which may be severe enough to stop the treatment. Further, the intravenous drug is expensive and requires pharmacy and nursing time for administration.

In view of the above, neither the oral nor intravenous iron therapy route is ideal and alternative routes of iron administration are desirable for dialysis patients.

Infusion of iron during dialysis is similar to an intravenous infusion, albeit at a slower rate. However, iron is known to be toxic when administered parenterally in its mineral form. The toxic effects may arise from precipitation of iron in the blood, producing multiple pulmonary and sometimes systemic emboli. Symptoms resembling that of fat embolism occur. Irritation of the gastrointestinal tract gives rise to diarrhea and vomiting. Also, depression of the central nervous system can lead to coma and death (29).

Very few noncolloidal iron compounds are suitable for intravenous administration. In the last five years, at least two groups of researchers have administrated ferric gluconate sodium intravenously for the treatment of iron deficiency in chronic hemodialysis patients (37,38). In these and various other studies, solubility, bioavailability and toxicity of various ferric compounds were shown to be different.

Recent studies have shown that polyphosphate compounds are possible candidates for intracellular iron transport (4,39). Among these polyphosphate compounds, pyrophosphate has been shown to be the most effective agent in triggering iron removal from transferrins (40-42). Pyrophosphate has also been shown to enhance iron transfer from transferrin to ferritin (5). It also promotes iron exchange between transferrin molecules (6). It further facilitates delivery of iron to isolated rat liver mitochondria (43).

The U.S. Patent 4,756,838 to Veltman, issued July 12, 1988, discloses a dry, free flowing, stable readily soluble, noncaking, particulate soluble products which are readily soluble in water and are useful for preparing solutions for use in hemodialysis. The patent discloses the fact that currently used dialysis procedures do not ordinarily take into account those materials in blood that are protein bound. Examples are iron, zinc, copper, and cobalt. The patent states that it is an object of the invention to make such materials as an integral part of dry dialysate products. However, no specific disclosure is made on how to make the iron available through the hemodialysis. No direction is given towards a noncolloidal iron compound as opposed to any other iron compound or mineral iron.

Ferric pyrophosphate has been used for iron fortification of food and for oral treatment of iron deficiency anemia (44). Ferric pyrophosphate has also been used for supplying iron to eukaryotic and bacterial cells, grown in culture (45). Toxic effects of ferric pyrophosphate have been studied by Mauer and coworkers in an animal model (48). This studied showed an LD_{50} slightly higher than 325 mg of ferric pyrophosphate per kilogram or approximately 35 milligrams of iron per kilogram body weight. The effective dose for replacing iron losses in hemodialysis patients is estimated to be 0.2 to 0.3 milligrams iron per kilogram per dialysis session. Therefore, the safety factor (ratio of LD_{50} to effective dose) is over 100.

Studies with another metal pyrophosphate complex, stannous pyrophosphate have reported immediate toxic effects. Since ferric iron forms a stronger complex to pyrophosphate than to stannous iron, or calcium ion, (50,51), hypocalcemia is not a known side affect of ferric pyrophosphate administration.

In view of the above, it is desirable to administer iron to a large proportion of dialysis patients by adding a soluble, non-colloidal iron compound to dialysis solutions, in order to replace ongoing losses of iron or to treat iron deficiency.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a method of administering iron in dialysis patients by infusion of a noncolloidal ferric compound, soluble in hemodialysis solutions during dialysis. The present invention further provides a pharmaceutical composition consisting essentially of a hemodialysis solution including a soluble, noncolloidal ferric compound. Preferably, the ferric compound is ferric pyrophosphate.

BRIEF DESCRIPTION OF THE FIGURES

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a graph showing serum iron per total iron binding capacity (TIBC) (percent);

Figure 2 is a pair of graphs showing serum iron versus time and iron per TIBC (percent) versus time.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a method of administration of iron in dialysis patients by infusion of a noncolloidal ferric compound, soluble in hemodialysis solutions, during dialysis. Administration can be to patients on hemodialysis (acute or maintenance) or peritoneal dialysis.

More specifically, as discussed above, hemodialysis patients are those patients undergoing hemodialysis for renal failure. Long-term hemodialysis therapy for treatment of end stage renal failure is referred to as maintenance hemodialysis. Patients on maintenance hemodialysis have been

estimated to lose about 2 to 3 grams of iron per year, corresponding to approximately 6 ml per day (2 liters per year) blood loss from all sources (11). These patients receive hemodialysis three times per week.

A specific example of a hemodialysis system is the Fresenius system. In the Fresenius system , the ratio of acid :bicarbonate:water:total is 1:1.23:32.77:35. Therefore, one part of the concentrated bicarbonate solution is mixed with 27.5 parts of the other (acid + water), to make the final dialysate. In order to make the bicarbonate concentrate (see Figure 3), purified water is pumped from the purified water source by a pipe (P) into a large tank (T). Fresenius supplied sodium bicarbonate powder packaged in plastic bags (B) and the contents of each bag are mixed with purified water in the tank, to make 25 gallons (94.6 liters) of bicarbonate solution. After thorough mixing with a stirrer (S) the concentrated solution is run into 20 liter plastic receptacles (R), that are capped. The concentrate is prepared within 24 hours of its use. Ferric pyrophosphate is freely soluble in the bicarbonate concentrate. Ferric pyrophosphate is pre-weighed and supplied packaged in plastic vials to the dialysis units. For a dialysate iron concentration of 4 μg/dl or FePyP concentration of 40 μg/dl, it can be calculated that bicarbonate concentrate should have a ferric pyrophosphate concentration of 40 x 27.5 = 1100 μg/dl, or 11 mg/liter. Therefore, 1040 mg of ferric pyrophospate added to 94.6 liter (25 gallons) of bicarbonate concentrate will generate dialysate with an iron concentration of 4 μg/dl.

Table 4. **Bicarbonate concentrates with a defined iron concentration achieved by addition of FePyP.**

Required Conc. of Fe in dialysate	Estimated Conc. of FePyP in dialysate	Estimated Amount of FePyP in concentrate
2 μg/dl	20 μg/dl	5.5 mg/L
4μg/dl	40 μg/dl	11 mg/L
8 μg/dl	80 μg/dl	22 mg/L
12 μg/dl	120 μg/dl	33 mg/L

Dialysate Fe concentration will be increased during the course of the study by adding different amounts of FePyP to the bicarbonate concentrate (Table 4). The technician preparing the concentrate would be required to add dry bicarbonate powder and ferric pyrophosphate crystals at the same time to purified water and the solution would be gently stirred until both compounds have dissolved resulting in a clear solution. Plastic receptacles (R) will be filled with bicarbonate concentrate containing iron and marked with the names of the study patients. This is to prevent patients not participating in the study from receiving dialysate iron therapy.

In a previous experiment, plasma (3.5 liters) was dialyzed using an F-80 dialyzer with the plasma flow rate set at 300 ml/min. and the dialysate flow rate 800 ml/min. Ferric pyrophosphate (420 mg) was added to 20 liters of bicarbonate concentrate and intermittently stirred for one hour prior to the dialysis. This was a clear solution with a light greenish yellow

tinge. The final dialysate was a clear, colorless solution, with 5 μg/dl iron content, as measured by a calorimetric assay. Physiological saline solution was added to the plasma every 15 minutes to compensate for obligate ultrafiltration, and to keep the plasma volume constant. Serum Fe and TIBC were measured at frequent intervals. There was a progressive increase in plasma iron concentration (A), and transferrin saturation (B), as shown in Figure 2.

By hemodialysis, it is meant that a hemodialyzer is used to remove certain solutes for the blood by virtue of their concentration gradients across a semipermeable membrane. Such membranes may be reused for a single patient. The hemodialyzer is an apparatus by which hemodialysis is performed, blood being separated by the semipermeable membrane from a solution of such composition as to secure diffusion of certain solutes from the blood. The hemodialyzer is popularly called an artificial kidney. The hemodialyzer can be used for ultrafiltration by which differences in fluid pressure bring about filtration of a protein-free fluid from the blood.

The hemodialysis solution of the present invention is characterized by including a noncolloidal ferric compound, preferably having a molecular weight of less than 1000 daltons. Most preferably the ferric compound should be 1) soluble in hemodialysis solutions in adequate concentrations; 2) efficiently transfer from the dialysate to the blood compartment; 3) bind to transferrin in the plasma and be available for use by

tissue; 4) be well tolerated without any short or long term side effects; and 5) be economical. In view of these parameters, ferric pyrophosate is the preferred compound for use with the present invention.

Ferric pyrophosate ($Fe_4O_{21}P_6$) has a molecular weight of 745.25. It is a nonahydrate, yellowish-white powder. It has been used as a catalyst, in fireproofing synthetic fibers and in corrosion preventing pigments.

In use, hemodialysis machines utilize an automated proportioning system to mix a sodium bicarbonate concentrate, an acid concentrate containing sodium chloride, potassium chloride, calcium chloride, sodium acetate, and glucose, and dionized water in specific proportions to generate the final dialysate solution. The dialysate concentrates are usually supplied by the manufactures either as a solution ready to use or as a premixed powder that is added to purified water in large reservoirs.

After thorough mixing, the bicarbonate concentrated solutions are packaged into approximately 20 liter plastic jugs. The concentrates acid to bicarbonate may be prepared the same day or a day prior to their use. The two concentrates are pumped into a chamber in the dialysis machine where they are mixed with purified water to make the final dialysate solution.

Generally, the ionic composition of the final dialysate solution is as follows: Na^+137-143 mEq/L, K^+0-4.0 mEq/L, Cl^- 100-111 mEq/L, Mg^2 0.75-1.5 mEq/L, HCO_3^- 30-35mEq/L, and acetate 2-4.5 mEq/L. The dialysate is pumped into the dialyzer at a flow rate of 0.5 to 1 liter per minute. After a

single pass through the dialyzer, the spent dialysate is pumped back into the dialysis machine from which it exits into the drain.

In accordance with the present invention, the ferric pyrophosphate is added to the final dialysate by its addition to one or both of the dialysate concentrates. Since the concentrates are diluted several fold in the machine by admixture with water, the compound has to be added a proportionally higher concentration in the concentrate or concentrates.

Preferably, 2 to 5μg of the ferric compound is infused per deciliters of the hemodialysis solution to a patient during a dialysis treatment. Accordingly, 4 to 50 milligrams of iron are infused into the patient during a two to five hour hemodialysis session to the patient.

In view of the above, the present invention provides pharmaceutical composition consisting essentially of the hemodialysis solution including a soluble noncolloidal ferric compound, preferably ferric pyrophosphate.

It must be recognized that various specific solutions are utilized to formulate hemodialysis solutions **it is a trademark or not).**

The following studies demonstrate the preparation and utility of the present invention.

EXPERIMENTS

The following experiments were performed to demonstrate the solubility of ferric pyrophosphate in a final dialysate solution and the dialysate concentrates.

Methods: Ferric pyrophosphate ($Fe_4(P_2O_7)_3$, M.W. 745.2, CAS 10058-44-3) (hereinafter PePyP) is a greenish yellow crystalline compound that is known to have a solubility of 50 mg per ml. in warm water (Catalog no. P 6526; Sigma Chemical Co., St. Louis, Missouri). Initially a small amount of FePyP crystals were added to the acid (pH, 2.49) and basic (pH, 7.81) concentrates and the dialysate (pH, 7.15). FePyP dissolved readily in the dialysate and the bicarbonate concentrate, forming a yellow-orange solution. However there was incomplete dissolution in the acid concentrate, and a precipitate was clearly visible. Since the concentrated bicarbonate

Table 2. Concentration of iron in bicarbonate concentrate after the addition of ferric pyrophosphate

Amount of FePyP added	Expected iron conc.	Measured conc. of Fe
2 mg/ml	0.2 mg/ml or 20 mg/dl	20.250 mg/dl
5 mg/ml	0.5 mg/ml or 50 mg/dl	40.660 mg/dl
10 mg/ml	1.0 mg/ml or 100 mg/dl	94.500 mg/dl
20 mg/ml	2.0 mg/ml or 200 mg/dl	206.500 mg/dl

*note – 10% of FePyP is Fe

solution is diluted several fold in the formation of the final dialysate, the concentration of FePyP in the bicarbonate concentrate should be appropriately higher than the desired dialysate concentration. Therefore solubility of FePyP in the bicarbonate concentrate was tested by adding variable amounts of FePyP and measuring the iron content of the mixture by the standard calorimetric method. The results are shown in Table 2. The measured and expected concentrations of iron were similar, showing that FePyP is highly soluble at the concentrations tested. A clear solution was formed within one hour of adding FePyP to the bicarbonate concentrate or the dialysate. Therefore, in dialysis practice, dialysate with a specific concentration of FePyP can be generated using a bicarbonate concentrate containing a proportionately higher concentration of FePyP.

In a second set of experiments, an *in vitro* dialysis of plasma, utilizing a conventional hemodialysis set up was used to show that the addition of even small amounts of ferric pyrophosphate to a dialysate solution results in significant transport of iron into the blood compartment during dialysis. This occurs because the transferred iron avidly binds to transferrin in the plasma.

Methods: Plasma was obtained from a uremic patient undergoing plasma exchange therapy for Goodpastures syndrome. Citrated plasma was stored at -20°C in plastic bags. In three separate experiments plasma was dialyzed against dialysates with different concentration of Fe, prepared by adding variable amounts of FePyP to the bicarbonate concentrate. Dialyzers

with a polysulfone membrane (Fresenius, USA) were used. When the volume of plasma being dialyzed was less than 1000 ml, a small dialyzer (F-4, Fresenius) with small blood volume (65 ml) and surface area (0.8 sq. meter) was used at a plasma flow rate of 100 ml/min. With a larger volume of plasma, a F-80 dialyzer with a priming volume of 120 ml and a surface area of 1.8 sq. meter was used at a plasma flow rate of 300 ml/min. Heparin (500 units per hour) was infused to prevent clotting in the circuit. Serum was drawn at regular intervals during the experiment and serum iron (Fe), total iron binding capacity (TIBC) and transferrin saturation (Fe/TIBC x 100) were measured by a calorimetric assay. The obligate ultrafiltration of fluid during hemodialysis was compensated by a continuous infusion of 0.9% saline. The iron parameters were corrected for net ultrafiltration by expressing the results as transferrin saturation.

Results: There was an increase in serum iron and transferrin saturation with time when iron was added to the dialysate (Figure 1). The increment in serum Fe and transferrin saturation was more as the concentration of iron in the dialysate was increased (Figure 1). There was a near doubling of transferrin saturation after two hours of dialysis with a dialysate iron concentration of 8 µg/dl (Figure 1).

Experimental parameters were then chosen to mimic conditions that prevail in actual dialysis practice. Therefore, 3.5 liters of plasma

(approximating the plasma volume in a 70 kg. patient) was dialyzed against a dialysate with 5μg/dl Fe concentration.

The hourly increase in plasma iron concentration was 23, 23, 35 and 45 μg/dl, and the net increase in iron concentration was 140 μg/dl over the course of the experiment. Therefore 5 mg iron (or ~50 mg FePyP) was infused into 3.5 liters of plasma, using a dialysate with 5 μg iron per dl. It should be noted that 420 mg of FePyP added to 20 liters of the bicarbonate concentrate imparted a very light greenish tinge to the solution, but after dilution with water the final dialysate was colorless.

In conclusion, ferric pyrophosphate can be added to the bicarbonate concentrate, aiming for an iron concentration of 2-50 μg/dl in the final dialysate depending on the degree of Fe deficiency. Hemodialysis with iron containing dialysate (Dialytic Iron Therapy) does result in transfer of iron to the blood compartment. In these *in vitro* experiments, maximum iron transfer cannot be obtained since transferrin is confined to a closed system. In vivo, the release of iron to the erythron and to the tissues by transferrin increases the total amount of iron that can enter the blood compartment. Therefore, dialysate iron therapy is a safe and effective route of iron delivery to hemodialysis patients.

In view of the above experiments , it is demonstrated that hemodialysis utilizing a hemodialysis solution containing iron compounds such as ferric pyrophosphate can be used to increase the amount of

bioavailable iron in a mammal. The data demonstrates that the ferric pyrophosphate is soluble in hemodialysis solutions in adequate concentrations, efficiently transfers from the dialysate to the blood compartment, and binds to the transferrin in the plasma. This data combined with previous studies showing the safety of ferric pyrophosphate demonstrates the utility of the present invention as a means for providing bioavailable iron in a mammal, especially a dialysis patient requiring parenteral iron administration.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically describe.

U.S. DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

THIS IS TO CERTIFY that page (s) 18 thru 22 *is missing from the U.S. Patent and Trademark Office microform records.*

Certifying Officer _____ *DATE* _____

What is claimed is:

1. A method of iron administration dialysis patients by infusion of a noncolloidal ferric compound, soluble in dialysis solutions, during dialysis.

2. A method as set forth in claim 1 wherein the ferric compound has a molecular weight of less than 5000 daltons.

3. A method as set forth in claim 2 wherein the ferric compound is ferric pyrophosphate.

4. A method as set forth in claim 1 wherein said infusion step is further defined as infusing up to 100 μg of the ferric compound per dl of the hemodialysis solution to the patient.

5. A pharmaceutical composition consisting essentially of a dialysis solution including a soluble, noncolloidal ferric compound.

6. A composition as set forth in claim 5 wherein said ferric compound has a molecular weight of less than 5000 daltons.

7. A composition as set forth in claim 6 wherein said ferric compound is ferric pyrophosphate.

8. A method of increasing the amount of bioavailable iron in a mammal by administering a noncolloidal ferric compound, soluble in dialysis solutions, during dialysis.

MAIL ROOM MAY 8 1997 51 PAT. OFF.

FORM 7-3

PATENT

Attorney's Docket Number: 1059.00017

Applicant or Patentee: Ajay Gupta

Serial or Patent No: _____

Filed or Issued: Herewith

For: _____

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)--NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: Henry Ford Health System
Organization Address: 1 Ford Place, 4B
Detroit, Michigan 48202-3450

Type of Organization:

___ University or other Institution of Higher Education

X Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)

___ Nonprofit Scientific or Educational under Statute of State of the United States of America

___ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3) if located in the United States of America (State: _____)
(Citation of Statute: _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and 41(b) of Title 35, of United States Code with regard to the invention entitled:

By Inventor(s): Ajay Gupta
Described in:

X the specification filed herewith.

___ application serial no. _____, filed _____.

___ patent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the the invention averring to their status as small entities. (37 CFR 1.27)

NAME: _____

ADDRESS: _____

___ Individual ___ Small Business ___ Nonprofit Organization

NAME: _____

ADDRESS: _____

___ Individual ___ Small Business ___ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Paul M. Shirilla
Title in Organization: System Vice Pres., Legal Affairs

Address of Person Signing: 1 Ford Place, 4B
Detroit, Michigan 48202-3450

SIGNATURE: Paul M. Shirilla **Date:** 12/9/96

FORM 7-3

MAIL ROOM
DEC
31
1996
PAT. & TRADEMARK OFF.

FORM 7-3

PATENT

Attorney's Docket Number: 1059.00017

Applicant or Patentee: Ajay Gupta

Serial or Patent No: _____

Filed or Issued: Herewith

For: _____

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(d)--NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: Henry Ford Health System
Organization Address: 1 Ford Place, 4B
Detroit, Michigan 48202-3450

Type of Organization:

___ University or other Institution of Higher Education

X Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)

___ Nonprofit Scientific or Educational under Statute of State of the United States of America

___ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3) if located in the United States of America (State: _____)
(Citation of Statute: _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and 41(b) of Title 35, of United States Code with regard to the invention entitled:

By Inventor(s): Ajay Gupta
Described in:

X the specification filed herewith.

___ application serial no. _____, filed _____.

___ patent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the the invention averring to their status as small entities. [37 CFR 1.27]

NAME: _____

ADDRESS: _____

___ Individual ___ Small Business ___ Nonprofit Organization

NAME: _____

ADDRESS: _____

___ Individual ___ Small Business ___ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Paul M. Shirilla
Title in Organization: System Vice Pres., Legal Affairs

Address of Person Signing: 1 Ford Place, 4B
Detroit, Michigan 48202-3450

SIGNATURE: Paul M. Shirilla **Date:** 12/9/96

08/755,954 12/02/96



PATENT

Attorney's Docket No. 1059.00017

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR CIP)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: *(check one applicable item below)*

X original
___ design
___ supplemental

NOTE: If the declaration is for an international Application being filed as a divisional, continuation or continuation-in-part application do not check next item; check appropriate one of last three items.

___ national stage of PCT

NOTE: If one of the follow 3 items apply then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR CIP.

___ divisional
___ continuation
___ continuation-in-part (CIP)

INVENTORSHIP IDENTIFICATION

WARNING: If the inventors are each not the inventors of all the claims an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

METHOD AND PHARMACEUTICAL COMPOSITION FOR REPLACING IRON LOSSES IN HEMODIALYSIS PATIENTS

SPECIFICATION IDENTIFICATION

the specification of which: (complete (a), (b) or (c))
(a) ___ is attached hereto.
(b) X was filed on 12-31-96 as US Serial No. 08/775,595 or
___ Express Mail No., as Serial No. not yet known _____ and was amended on _____ (if applicable).

(c) ___ was described and claimed in PCT International Application No. ___ _____ filed on _____ and as amended under PCT Article 19 on ___ _____ (if any).

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations. § 1.56(a).

___ In compliance with this duty there is attached an information disclosure statement 37 CFR 1.97.

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) _X_ no such applications have been filed.

(e) ___ such applications have been filed as follows

NOTE: Where item (c) is entered above and the International Application which designated the U.S. claimed priority check item (e), enter the details below and make the priority claim.

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIM UNDER 35 USC 119
			___ YES ___ NO
			___ YES ___ NO
			___ YES ___ NO
			___ YES ___ NO
			___ YES ___ NO

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

Kenneth I. Kohn Reg. No. 30,955
Ilene N. Montgomery Reg. No. 38,972

SEND CORRESPONDENCE TO

Kenneth I. Kohn
Kohn & Associates
30500 Northwestern Hwy
Suite 410
Farmington Hills, MI 48334

DIRECT TELEPHONE CALLS TO:
(NAME AND TELEPHONE NUMBER)

Kenneth I. Kohn
(810) 539-5050

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

Full name of sole or first inventor Ajay Gupta

Inventor's signature _____

Date 4-21-97 Country of Citizenship Indian

Residence Farmington Hills, Michigan 48331

Post Office Address 39151 Horton

Farmington Hills, Michigan 48331

Full name of second joint inventor, if any _____

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

CHECK PROPER BOX(ES) FOR ANY OF THE FOLLOWING ADDED PAGE(S) WHICH FORM A PART OF THIS DECLARATION

___ Signature for third and subsequent joint inventors. Number of pages added ___

___ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added ___

___ Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. Number of pages added ___

* * *

___ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (CIP) application.

___ Number of pages added ___

* * *

___ Authorization of attorney(s) to accept and follow instructions from representative.

* * *

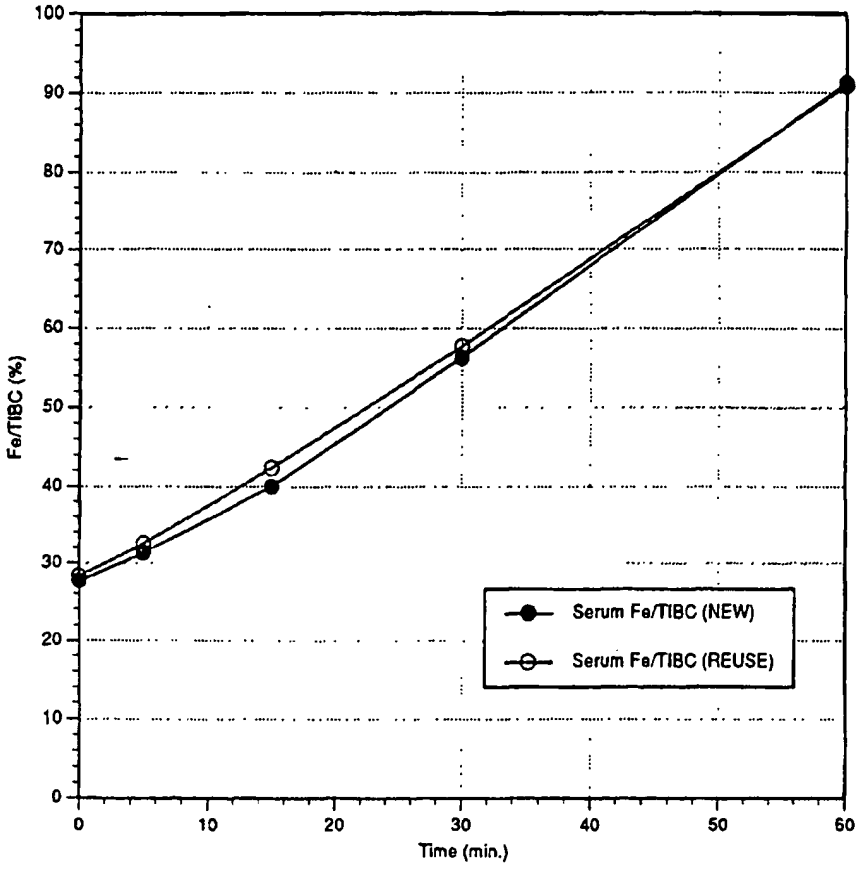
If no further pages form a part of this Declaration then end this Declaration with this page and check the following item

X This declaration ends with this page.

PATENT 5555/60

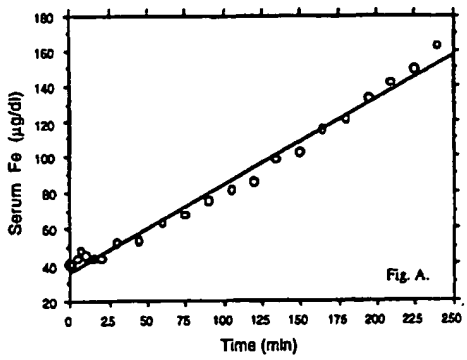
08/775595

Iron flux during dialysis using a new vs. reused membrane



Data from: Henry Ford Hospital 10/96

FIG 1



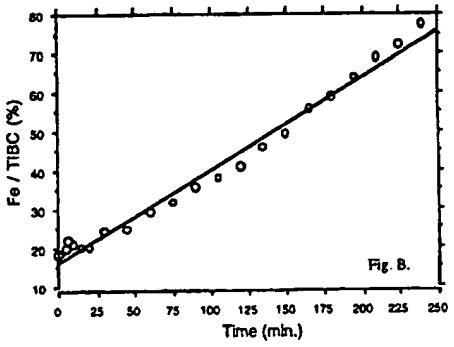


Fig 2

08/775595 - 12-31-96